gel, with 3 mA current for tube[4]. For the determination of AP activity zone, the gel was squeezed out of the tubes following electrophoresis, and divided into 8 segments 6 mm each. The corresponding segments from more tubes were pooled, homogenized in phosphate buffer pH 7.0 (0.05 M) and the activity was followed in the eluates (substrate Leu-, Ala-, CH_3-Cys-, Gly-Phe- and Gly-Leu-p-NA). The fraction containing higher-molecular AP was divided in 4 proteinic zones with AP activity localized on a single site near the start. In the fraction with lower-molecular AP divided in 7 proteinic zones, the AP activity migrated 18–25 mm in separation gel. In these fractions, free from the NH_2-dipeptidyl hydrolase activity, the course of stepwise hydrolysis Gly-Phe- and Gly-Leu-p-NA was the same as shown in figure 2. Thus we can exclude the possibility that in this effect there participate some peptidases other than those studied.

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Changes in the activities of protein kinase modulators in the cerebellum of mice due to ethanol, caffeine, or phenobarbital administration[1]

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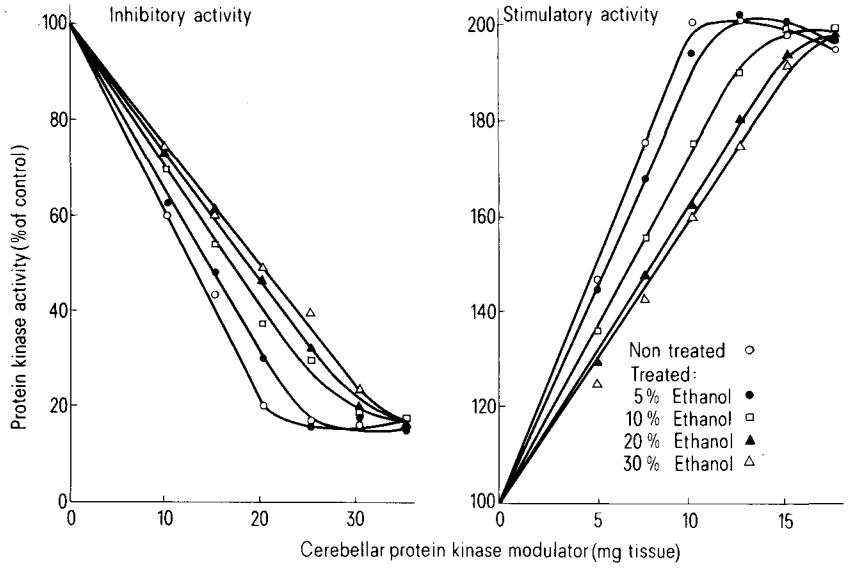
Summary. Decreased activities of both the inhibitory modulator of adenosine 3′:5′-monophosphate (cAMP)-dependent protein kinase (A-PK) as well as the stimulatory modulator of guanosine 3′:5′-monophosphate (cGMP)-dependent protein kinase (G-PK) from the mouse cerebellum were noted due to the administration of excessive doses of ethanol, caffeine, and phenobarbital for up to 28 days. The dose-dependence of the inhibition of A-PK or the stimulation of G-PK was observed as a function of the amount of protein kinase modulators in the cerebellum of mice receiving different doses of ethanol.

Recently, we have reported that the inhibitory modulator of A-PK and the stimulatory modulator of G-PK are separated proteins which exist as a mixture in crude protein kinase modulator preparation obtained from mammalian tissues[2-5]. The inhibitory modulator appears to be the same as the protein inhibitor of Walsh et al.[6]. It was reported that the level of inhibitory modulator in rabbit heart decreased due to starvation and alloxan treatment[7]. Skala et al. showed that inhibitory modulator in brown rat adipose tissue was highest perinatally and that it declined 10 days after birth[8]. Moreover, it was found that in diabetic rats the inhibitory and stimulatory activities of the modulators from the pancreas were higher, whereas those from the fat were lower[9]. We were able to show the suppression of modulators in the liver of mice due to ethanol, caffeine, or phenobarbital administration in recent studies[10], but there still remains to be explored the possible relation between the physiological and toxicological effects of these drugs on the 2 activities of the modulators in the mouse cerebellum.

Materials and methods. 8 groups of young adult male ICR mice (mean b.wt, 19.1±2.0 g) were used. The drinking water contained 5% sucrose and one of the following drugs: ethanol (5%, 10%, 20% and 30%, v/v) caffeine (0.5 mg/ml), phenobarbital (1.0 mg/ml), and vitamin C (1.0 mg/ml) respectively. Each group contained 15 - 25 mice, and the duration of drug administration was 2 or 4 weeks. The drugs were omitted from the drinking water for the control mice. After the animals were killed by decapitation, the cerebellum was removed immediately and homogenized with 5 vol. of ice cold 5 mM potassium phosphate buffer,



The inhibition of A-PK and the stimulation of G-PK as a function of the amount of protein kinase modulators from the cerebellum of mice treated with different doses of ethanol for four weeks. Each point shown represents mean of the values obtained with triplicate samples.

pH 7.0, using a glass-teflon homogenizer. Protein kinase modulator was prepared from the extracts through the steps of boiling and tricholoroacetic acid-precipitation[2-6].

cAMP-dependent protein kinase from bovine hearts was purified through the DEAE-cellulose step[11]. cGMP-dependent protein kinase from mouse lungs was purified from the step of Sephadex G-200 gel filtration[12]. The standard assay system[2-5,12] for both A-PK and G-PK contained, in a final volume of 0.20–0.25 ml, potassium phosphate buffer, pH 7.0, 10 µmoles; theophylline, 0.5 µmoles; arginine-rich histone (HA, Worthington), 40 µg; $MgCl_2$, 2 µmoles; [γ-^{32}P] ATP (New England Nuclear), 1 nmole, containing about 0.9×10^6 cpm; either cAMP or CGMP. 80 pmoles; appropriate amounts of protein kinases, 20–40 units; with or without protein kinase modulators, up to 110 µg of protein, prepared from 35 mg of fresh tissue. The reaction was carried out for 10 min at 30 °C. 1 unit of protein kinase activity is defined as that amount of enzyme that transferred 1 pmole of ^{32}P from [γ-^{32}P] ATP to recovered histone under the assay conditions. 1 unit of the inhibitory activity of the modulators is defined as that amount of the factor that depressed 20% of 20 units of A-PK in the presence of 0.4 µM cAMP. 1 unit of stimulatory activity of the modulators is defined as that amount of the factor that augmented 20% of 20 units of G-PK in the presence of 4 µM cGMP.

Results. The changes in the activities of modulators from the cerebellum of mice treated with various drugs are shown in the table. The degree of suppression of both modulators was similar for groups treated with caffeine (0.5 mg/ml) and phenobarbital (1.0 mg/ml). The decrease in the stimulatory modulator, however, was much greater than that of the inhibitory modulator in the ethanol-treated group. The decrease in both modulator activities in mice treated for a shorter period (24 h) with these drugs was also noted (data not shown). A sedative-hypnotic obtained from DePree, each tablet providing 25 mg of methapyriene HCl, 0.25 mg of scopolamine aminoxide HBr, and 200 mg of salicylamide, was also shown to have similar effects to those of phenobarbital at the concentration of 1.0 mg/ml (data not shown). No further suppression of the modulator activities was observed with higher doses and/or prolonged duration of administration of caffeine or phenobarbital. Vitamin C was shown to have no significant effect on the changes of activities of both modulators. Inclusion of sucrose (5%) in drinking water was found to have no effect on the modulator activities (data not shown). The amount of water consumed was the same for all groups, and it was 1.7 ± 0.2 ml/mouse/day.

A linear relationship was noted between the inhibition of A-PK or the stimulation of G-PK, and the amount of modulators in the cerebellum of the ethanol-treated animals (figure). A reduced inhibition and stimulation was observed by increasing ethanol concentration up to 30% (v/v).

Discussion. The present data indicate that not only certain stimulants (such as ethanol and caffeine), but also certain depressors (such as phenobarbital) of the CNS suppressed both inhibitory and stimulatory modulators of protein kinases in the mouse cerebellum when the animals were excessively treated with these drugs. The present studies in the cerebellum coupled with findings in other tissues as reported by others[7-9], suggest a role for these 2 factors in certain toxicological as well as physiological processes. Several reports have been made on the inhibition of cyclic nucleotide phosphodiesterases by methylxanthines[13,14]; the inhibition of guanylate cyclase by ethanol[15]; whereas the stimulation of adenylate cyclase by ethanol[16]; as well as the induction of A-PK by phenobarbital[17]. Furthermore, our preliminary studies also indicate the changes in the activities of both A-PK and G-PK due to alcohol administration (unpublished data). In addition to that another heat-stable inhibitory modulator (protein inhibitor) for both A-PK and G-PK recently reported by Szmigielski et al.[18], we found yet another species of inhibitory modulator with similar properties but different molecular weight and/or shape[19]. These findings gradually reveal the complexitiy of the cyclic nucleotide system. In spite of the above evidence[13-17] suggesting that the normal regulation of cyclic nucleotide system may be impaired by those drugs mentioned above, more basic information on all related enzymes and regulatory factors will be required in order to define the role of the modulators of protein kinase in the pathophysiology.

Changes in the activities of modulators from the cerebellum of mice due to administration of ethanol, caffeine, phenobarbital, or vitamin C

Administration		Modulator activity (units/g tissue)	
Agent	Days	Inhibitory	Stimulatory
None (control)	0	198.0± 8.5	529.9± 9.8
Ethanol (30%, v/v)	14	127.2± 4.6[b]	299.3±32.1[b]
	28	114.4± 7.1[b]	258.9±24.6[b]
Caffeine (0.5 mg/ml)	14	148.5± 4.9[b]	368.9±10.9[b]
	28	125.3±10.8[b]	313.2±31.1[b]
Phenobarbital (1.0 mg/ml)	14	136.1±27.3[a]	373.4±30.7[b]
	28	120.9±20.1[b]	276.3±18.5[b]
Vitamin C (1.0 mg/ml)	14	196.2± 9.7	523.5± 7.9
	28	197.3± 8.1	522.1± 9.5

Assay conditions were as described in the text. The means (± SE) of the values obtained from 3 to 9 samples for each group were shown. Significantly different from control animals: [a] $p<0.05$; [b] $p<0.01$.

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